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Longitudinal Changes in Multiple Biomarkers Are Associated with Cardiotoxicity in Breast Cancer Patients Treated with Doxorubicin, Taxanes, and Trastuzumab

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Abstract

BACKGROUND—Biomarkers may play an important role in identifying patients at risk for cancer therapy cardiotoxicity. Our objectives were to define the patterns of change in biomarkers with cancer therapy and their associations with cardiotoxicity.

METHODS—In a multicenter cohort of 78 breast cancer patients undergoing doxorubicin and trastuzumab therapy, 8 biomarkers were evaluated at baseline and every 3 months over a maximum follow-up of 15 months. These biomarkers, hypothesized to be mechanistically relevant to cardiotoxicity, included high-sensitivity cardiac troponin I (hs-cTnI), high-sensitivity C-reactive protein (hsCRP), N-terminal pro–B-type natriuretic peptide (NT-proBNP), growth differentiation factor 15 (GDF-15), myeloperoxidase (MPO), placental growth factor (PlGF), soluble fms-like

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tyrosine kinase receptor-1 (sFlt-1), and galectin 3 (gal-3). We determined if biomarker increases were associated with cardiotoxicity at the same visit and the subsequent visit over the entire course of therapy. Cardiotoxicity was defined by the Cardiac Review and Evaluation Criteria; alternative definitions were also considered.

RESULTS—Across the entire cohort, all biomarkers except NT-proBNP and gal-3 demonstrated increases by 3 months; these increases persisted for GDF-15, PlGF, and hs-cTnI at 15 months. Increases in MPO, PlGF, and GDF-15 were associated with cardiotoxicity at the same visit [MPO hazard ratio 1.38 (95% CI 1.10–1.71), $P = 0.02$; PlGF 3.78 (1.30–11.0), $P = 0.047$; GDF-15 1.71 (1.15–2.55), $P = 0.01$] and the subsequent visit. MPO was robust to alternative outcome definitions.

CONCLUSIONS—Increases in MPO are associated with cardiotoxicity over the entire course of doxorubicin and trastuzumab therapy. Assessment with PlGF and GDF-15 may also be of value. These findings motivate validation studies in additional cohorts.

The use of anthracyclines and trastuzumab (Herceptin®) have led to improvements in breast cancer survival but are also associated with an increased risk of cardiovascular toxicity, including left ventricular ejection fraction (LVEF) decline,[7] cardiomyopathy, and heart failure (HF) (1, 2). Trastuzumab is associated with a 2.5-fold increased risk of cardiotoxicity, and the risk is even higher with prior anthracycline exposure (3, 4). Despite the severity of the problem, however, few diagnostic tools can be used to identify or predict which patients will experience cardiotoxicity. Recognizing at-risk patients before potentially irreversible cardiac injury or overt HF could lead to the early initiation of cardioprotective strategies, prevent dose interruptions and delays in cancer therapy, and reduce cardiovascular and oncologic morbidity.

Some of the first studies of biomarkers in cardiooncology demonstrated that individuals with increases in cardiac troponin and natriuretic peptides during chemotherapy were more likely to experience cardiotoxicity over the subsequent 12 months (5–7). Recently, we reported an association between early increases (baseline to 3 months) in high-sensitivity cardiac troponin I (hscTnI) and myeloperoxidase (MPO) in breast cancer patients receiving anthracyclines and trastuzumab and the risk of a first cardiotoxic event (8). That study evaluated the changes in biomarkers exclusively during anthracycline chemotherapy and considered the only first cardiotoxic event for each individual patient. Here, we add to our earlier findings by examining whether increases in potentially mechanistic biomarkers across the entire course of anthracycline and trastuzumab therapy are associated with a cardiotoxic event, also evaluated over the entire course of therapy.

To address this question, we studied the temporal changes in 8 biomarkers measured at 3-month intervals during doxorubicin and trastuzumab therapy, for a maximum follow-up time of 15 months, and defined their associations with cardiotoxicity, occurring either concurrently or at a subsequent visit. These biomarkers were particularly relevant, as there is

[7]Nonstandard abbreviations: LVEF, left ventricular ejection fraction; HF, heart failure; hscTnI, high-sensitivity cardiac troponin I; MPO, myeloperoxidase; hsCRP, high-sensitivity C-reactive protein; GDF-15, growth differentiation factor 15; NT-proBNP, N-terminal pro–B-type natriuretic peptide; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase receptor 1; gal-3, galectin 3; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; HERA, Herceptin Adjuvant Trial.

evidence suggesting they reflect key aspects of the diverse biologic perturbations that occur with doxorubicin and trastuzumab cardiotoxicity (9–11). Proposed mechanisms of cardiotoxicity exist, with some studies suggestive of an important role for oxidative stress, inflammation, and vascular remodeling (11–13). Human clinical studies also demonstrate that cardiac injury and abnormal neurohormonal activation occur with these agents (5–7). Our panel thus included the following pathways and markers: cardiomyocyte injury (hs-cTnI); inflammation [high-sensitivity C-reactive protein (hsCRP) and growth differentiation factor 15 (GDF-15)]; neurohormonal activation [N-terminal pro–B-type natriuretic peptide (NT-proBNP)]; oxidative stress (MPO); antiangiogenesis and vascular remodeling [placental growth factor (PlGF) and soluble fms-like tyrosine kinase receptor 1 (sFlt-1)]; and fibrosis [galectin 3 (gal-3)].

Materials and Methods

STUDY POPULATION

Eligible patients were ≥18 years of age, diagnosed with human epidermal growth factor receptor 2 (HER2)-positive breast cancer, and planned for adjuvant therapy with an anthracycline-containing regimen followed by taxanes and trastuzumab. Patients were recruited from 4 institutions as previously described (8, 14). Patients with baseline LVEF <50% or those unable to provide informed consent were excluded.

Six planned study visits occurred at baseline before chemotherapy and every 3 months during chemotherapy over 15 months (Fig. 1). Demographics and clinical history were recorded at baseline. Transthoracic echocardiograms were obtained at each visit, and blood samples were obtained immediately before cancer therapy infusion at 3-month intervals. Consistent with all previous analyses, the primary cardiotoxicity end point was defined by use of the Cardiac Review and Evaluation Committee definition: a reduction in LVEF of ≥5% to <55% with symptoms of HF or an asymptomatic reduction in LVEF of ≥10% to <55% (15). In sensitivity analyses, an alternative definition of cardiotoxicity including a reduction in LVEF ≥10% to <50% was also evaluated (16).

All participants provided informed consent. The study protocol was approved by the institutional review boards of the participating institutions.

ECHOCARDIOGRAPHY

Transthoracic echocardiograms were acquired by use of Vivid 7 or E9 (GE Healthcare) and analyzed on the EchoPAC workstation (GE Healthcare) (8, 17). Left ventricular end-diastolic and end-systolic volumes were measured by the Simpson method of discs (18) in the apical 4- and 2-chamber views, and LVEF was calculated as follows: (end-diastolic volume – end-systolic volume)/enddiastolic volume × 100%. All measurements were made at a centralized reading center by a single observer who was blinded to all clinical and biomarker data.

BIOMARKERS

Venous blood was collected in standard tubes for plasma with both EDTA and lithium heparin, processed at 3100 rpm for 15 min, divided into aliquots, and stored at −80 °C until the time of assay. Measurement of hs-cTnI, a research prototype assay, and NT-proBNP were performed on the Dimension Vista 500 Intelligent Laboratory System (Siemens Healthcare Diagnostics); hsCRP was measured with a standard Architect immunoassay (Abbott Laboratories); and GDF-15, MPO, PlGF, sFlt-1, and Gal-3 were measured with prototype Architect chemiluminescent 2-step microparticle-based immunoassays (Abbott Laboratories). All measurements were performed on previously unthawed samples. CVs for all assays were <10%. Briefly, hs-cTnI had a 10% CV at 3 ng/L; NT-proBNP <3%; hsCRP ≤6%; GDF-15 ≤4.5%; MPO ≤9.8%; PlGF ≤6.7%; sFlt-1 ≤5.9%; and gal-3 ≤4.5%. Further details are in the Supplemental File, which accompanies the online version of this article at http://www.clinchem.org/content/vol61/issue9.

STATISTICAL METHODS

Each biomarker was recorded as the ratio of the value at each visit relative to baseline, and, for the purpose of modeling, transformed to a $\log_2$ scale. Here each unit increase in the biomarker ratio represents a doubling in value compared to baseline. Using a Wilcoxon signed rank, we determined whether the median of the ratio (original scale) differed from 1.0 at each time point; the hypothesis tests were Bonferroni corrected for each bio-marker. Individual trajectories of biomarker ratios were visualized with a locally smoothed mean.

As in clinical practice, we evaluated the cardiotoxicity outcome at each visit and defined this as present or absent. Presence of cardiotoxicity at an individual visit reflected either new or persistent cardiotoxicity. We first modeled the outcome as a function of the individual biomarker ratio at the same visit (i.e., both the ratio and cardiotoxicity at the 3-month visit, both the ratio and cardiotoxicity at the 6-month visit, and so forth to 15 months) (Fig. 1). Next, we evaluated the association between each biomarker ratio and cardiotoxicity at the subsequent 3-month visit (i.e., the bio-marker ratio at the 3-month visit with cardiotoxicity at the 6-month visit, the biomarker ratio at the 6-month visit with cardiotoxicity at the 9-month visit, and so forth to 15 months). The models were implemented by use of a repeated-measures Cox model with the biomarker ratios entered into the model as time-varying covariates. Information from all visits was incorporated into a single estimated risk and expressed as a hazard ratio (HR) per doubling of the biomarker. Significance was evaluated with a robust Score test ($P < 0.05$).

We then constructed a multiple biomarker model that included biomarkers with a P value <0.20 in individual models, retaining only those with $P < 0.05$. For the multivariable model, the robust Score test was again used to test global significance. Wald tests were used to evaluate individual terms (19). To consider the potential clinical significance of the biomarkers, we determined the 75th and the 90th percentiles of the ratios for the bio-markers included in the final multivariable model. For each time point, we then determined the relative risk of cardiotoxicity for the 2 percentiles compared to the median biomarker ratio.

We carried out 2 types of sensitivity analyses. To address alternative definitions of cardiotoxicity within the field of cardio-oncology, we repeated the modeling procedure with the individual covariates but with the Herceptin Adjuvant Trial (HERA) outcome (reduction in LVEF ≥10% to <50%). To address possible bias in our estimates due to incomplete data, we constructed a dataset in which we used the median across all patients at each time point to impute the biomarker concentrations of individuals with missing data and excluded the 15-month visit, where there was a high rate of missing data. We constructed an additional dataset in which we excluded a site where the missing data rates were high. For both datasets, we repeated the analyses described above.

A priori, hypothesis tests were specified to be 1-sided, given findings from our prior work (8), where only increases in biomarkers were relevant in defining cardiotoxicity risk. For a 1-sided type 1 error rate of 0.05, a sample size of 78 patients, and an event rate of either 15% or 30%, a time-to-first-event Cox model would have 80% power to detect relatively large HRs, on the order of 1.7–2.1, respectively (PASS 2008). In a repeated-measures Cox model, the detectable HRs are expected to be slightly lower. Analyses were performed with R 3.03 (R Development Core Team).

Results

STUDY POPULATION

The mean age of these 78 women was 49 years, and the mean body mass index was 24.5 kg/m^2 (Table 1). At time of enrollment, 27% had hypertension, 23% had hyperlipidemia, and 9% smoked. At baseline, the mean (SD) LVEF was 64% (5%).

PATTERNS OF CARDIOTOXICITY DURING DOXORUBICIN AND TRASTUZUMAB THERAPY

Over the course of the 15-month study, 23 patients experienced 39 events. Twelve patients had a single event, 7 had 2, and 4 had >2 events; these repeated events primarily reflected a persistent LVEF decline. Rates of cardiotoxicity were <5% at 3 months and ranged from 9.3% to 19.1% for the remainder of the study. The first evidence of cardiotoxicity occurred most frequently at 6 and 12 months. After the diagnosis of cardiotoxicity, trastuzumab was discontinued in 3 patients; 1 was treated with angiotensin-converting enzyme inhibitors and beta-blockers; no additional intervention was performed in the other 2 patients.

BIOMARKER CHANGES DURING DOXORUBICIN AND TRASTUZUMAB THERAPY FOR THE ENTIRE COHORT

Baseline concentrations of each biomarker appear in Table 2. For the cohort as a whole, all biomarkers except gal-3 and NT-proBNP showed significantly increased concentrations at 3 months compared with baseline (Table 2, online Supplemental Fig. 1). At subsequent visits, the concentrations tended to decline, with only GDF-15, PlGF, and hs-cTnI demonstrating persistently increased concentrations at 15 months. As shown in Table 2, the median concentration for GDF-15 at baseline was 500 ng/L (interquartile range 349–733). Compared with baseline, the biomarker concentration at 3 months was 1.6-fold greater, decreasing to 1.2-fold at 15 months. In contrast, changes in MPO and PlGF concentrations were less pronounced. The median ratio of these 2 biomarkers increased to 1.3 at 3 months,

and then decreased to a ratio of 0.9–1.1 by 15 months. Across patients, MPO showed the largest variation; PlGF showed the smallest variation, with PlGF also demonstrating moderate to strong correlations ($P > 0.50$) across all visits.

Interestingly, the median hs-cTnI concentration at baseline was 1.3 ng/L; concentrations increased to a median of 9.9-fold that of baseline at 3 months and 12.0 at 6 months (Table 2, online Supplemental Fig. 1). In contrast to all other biomarkers, at 15 months, the hs-cTnI ratio still remained substantially increased and was 3.9-fold greater than baseline.

ASSOCIATION BETWEEN CHANGES IN INDIVIDUAL BIOMARKERS AND CARDIOTOXICITY AT THE SAME VISIT

The association between increases in individual biomarkers, as measured by the $\log_2$ biomarker ratio, and concurrent cardiotoxicity was estimated with a model that evaluated biomarker increases at 3 months and cardiotoxicity at 3 months, increases at 6 months and cardiotoxicity at 6 months, and so forth. As shown in Table 3, increases in MPO, PlGF, and GDF-15 were each individually associated with an increased risk of cardiotoxicity [MPO HR 1.37 (95% CI 1.11–1.69), $P = 0.02$; PlGF 3.77 (1.43–9.89), $P = 0.04$; GDF-15 1.80 (1.20–2.69), $P = 0.007$]. hsCRP was of marginal significance ($P = 0.07$), and hs-cTnI ($P = 0.30$) and NT-proBNP ($P = 0.19$) were not associated with cardiotoxicity.

In sensitivity analyses, findings were similar when we considered outcomes from 6 months onward during trastuzumab therapy alone (data not shown). When we considered alternative definitions of cardiotoxicity (reduction in LVEF ≥10% to <50% as described in HERA (16)), our findings were similar, with widely overlapping CIs (see online Supplemental Table 1). Here, there were only 11 events, and as a result, the power to detect significant effects was lower than in the analyses described above. Importantly, in our univariable models, increases in MPO [HR 1.72 (95% CI 1.26–2.36), $P = 0.04$] remained significantly associated with cardiotoxicity, and increases in PlGF approached significance [HR 3.32 (95% CI 0.96–11.56), $P = 0.06$]. The association between GDF-15 and cardiotoxicity was attenuated.

Our second set of sensitivity analyses addressed the issue of incomplete data. At the 3-, 6-, and 9-month visits, the proportion of incomplete biomarker data ranged from 8% to 13% for all biomarkers except hscTnI; for hs-cTnI, incomplete data rates were 15%–19% at these visits. At 12 months, these rates were 19% (all biomarkers but NT-proBNP or hs-cTnI) and 26%–30% (NT-proBNP and hs-cTnI). At 15 months, these rates increased to 33% and 51%–54%, respectively.

Importantly, for the question of bias, 1 site in particular had high rates of missing data that reflected logistical issues with the site, rather than specific patient characteristics. Excluding patients from this site and considering baseline, 3-, 6-, and 9-month visits, the percentage of missing biomarker data was only 5% (all biomarkers but NT-proBNP and hs-cTnI), 5%–12% (NT-proBNP), and 15%–19% (hs-cTnI). At 12 months, data were missing in 11% (all biomarkers but NT-proBNP and hs-cTnI) and 21%–23% (NT-proBNP and hs-cTnI) of patients. At 15 months, data were missing in 15% (all biomarkers but NT-proBNP and hs-cTnI) and 38%–41% (NT-proBNP and hs-cTnI).

To explore the potential impact of these incomplete data on the associations described above, we imputed the missing data by use of the median concentration across individuals from all sites. We then excluded the 15-month time point and also the 1 site with high rates of missing data. For these datasets, our findings were largely similar for GDF-15, MPO, and PlGF, with statistical significance maintained for these 3 markers (see online Supplemental Table 2). No substantive changes were observed in the results for any other biomarker.

ASSOCIATION BETWEEN CHANGES IN INDIVIDUAL BIOMARKERS AND CARDIOTOXICITY AT THE SUBSEQUENT VISIT

Next, we examined the association between changes in biomarkers as measured by the $\log_2$ biomarker ratio and cardiotoxicity at the subsequent visit. The goal of these models was to gain insight into cardiotoxicity prediction within a short time window. We found similar results for MPO ($P = 0.003$) and GDF-15 ($P = 0.02$), with a comparable, albeit not statistically significant, effect for PlGF ($P = 0.08$) (Table 3). Of note, gal-3 was also significantly associated with cardiotoxicity at the subsequent visit [HR 1.60 (95% CI 1.12–2.28), $P = 0.04$].

When we considered alternative definitions of cardiotoxicity as part of a sensitivity analysis (reduction in LVEF ≥10% to <50% (16)), our findings were similar (see online Supplemental Table 1). In sensitivity analyses described above, where we imputed the data, the results were also very similar (see online Supplemental Table 3).

ASSOCIATIONS BETWEEN CHANGES IN MULTIPLE BIOMARKERS AND CARDIOTOXICITY

Our multivariable models initially included all biomarkers with $P < 0.20$ in our univariable associations (MPO, PlGF, GDF-15, gal-3, NT-proBNP, and hsCRP). For the model with the biomarkers and outcome measured at the same visit, the final model (overall $P = 0.007$) included MPO, PlGF, and GDF-15. Risk estimates for the multivariable model were similar to those seen in the univariable models (Table 3). Associations between these 3 markers in combination and cardiotoxicity at the subsequent visit were also significant (overall $P = 0.02$). For both models, the results were very similar after imputation of the incomplete data, as shown in online Supplemental Tables 2 and 3 (overall $P = 0.02$).

Although our models yielded estimates of risk per doubling of each biomarker, we note that the potential clinical importance of a biomarker depends not only on this risk estimate, but also on the distribution of the ratio at each time point. For example, if 2 biomarkers are associated with the same risk, but 1 biomarker has more frequent doublings occurring than the other, the first potentially has more clinical impact. Thus, Fig. 2 considers the predicted risk of cardiotoxicity for MPO, PlGF, and GDF-15 at the 75th and 90th percentiles of the biomarker relative to the median biomarker ratio at the same visit (Fig. 2A) and the subsequent visit (Fig. 2B). In each figure, the risk relative to the median biomarker ratio at that time point is plotted. For both models, increases in MPO and GDF-15 at the 75th and 90th percentiles were associated with increases in cardiotoxicity risk of 1.1–1.6 (MPO) and 1.2–1.6 (GDF-15) compared with the median value for that biomarker. For PlGF, the increases were on the order of 1.1–1.3 at the 75th and 90th percentiles across the visits.

Discussion

This study of breast cancer patients is one of the first to explore the association between increases in multiple bio-markers and cardiotoxicity risk over the entire duration of doxorubicin followed by trastuzumab therapy. Our previous work (8) examined the association between early changes in biomarkers during doxorubicin therapy only (i.e., 3 months) and the first occurrence of cardiotoxicity. That study identified an association between an increase at 3 months in hs-cTnI and MPO and the risk of first cardiotoxicity (8). In the present study, we considered repeated biomarker increases for up to 15 months and their associations with each cardiovascular event, at either the same visit or the subsequent visit 3 months later. Importantly, our present work suggests that increases in MPO beyond 3 months remain a predictor of cardiotoxicity risk over the duration of doxorubicin/trastuzumab therapy. In addition, we identify 2 other candidate biomarkers, GDF-15 and PlGF, of possible interest. The independence of these markers in our multivariable model suggests additive utility.

Before this study, the association and time sequence between the development of cardiotoxicity and the increases in biomarkers over the entire course of doxorubicin and trastuzumab therapy was unclear. This motivated the 2 sets of models, 1 relating the biomarker concentration and cardiotoxicity at a concurrent visit, and 1 relating the biomarker concentration and cardiotoxicity at a subsequent 3-month visit. The similarity of our results for both models suggests that the associations are temporally robust. With an alternative definition of cardiotoxicity, our findings were similar.

Importantly, this study goes beyond our previous work and indicates that increases in MPO throughout the course of doxorubicin/trastuzumab therapy are significantly associated with cardiotoxicity. MPO is an enzyme secreted by polymorphonuclear leukocytes that is proatherogenic and prooxidant (20). It is believed to be a marker of oxidative stress (20), 1 of the central mechanisms of doxorubicin cardiotoxicity (21), as well as potentially related to ErbB2 inhibition by trastuzumab (22, 23). In our study, it is also possible that the MPO increases from doxorubicin persisted into the period when trastuzumab was administered. Overall, this study adds weight to our previous finding of MPO as a strong candidate biomarker of cardiotoxicity in breast cancer patients treated with doxorubicin and/or trastuzumab.

We identified 2 additional markers that may be relevant to doxorubicin and trastuzumab cardiotoxicity: PlGF and GDF-15. PlGF is a member of the vascular endothelial growth factor family and may play an important cardioprotective role through cardiomyocyte and endothelial cell paracrine signaling and promotion of angiogenesis (24, 25). As such, it is plausible that PlGF may be specifically relevant to the pathophysiology of anthracycline and trastuzumab cardiotoxicity, as data suggest these agents affect angiogenesis (26, 27). Circulating PlGF has shown to be of prognostic value in preeclampsia, acute coronary syndromes, and ischemic cardiomyopathy (28–31).

GDF-15 is a member of the transforming growth factor-β cytokine superfamily, expressed by multiple cell types (32, 33), and released in response to ischemia, injury, mechanical

stretch, oxidative/nitrosative stress, and inflammation (34). As a biomarker, GDF-15 has been associated with increased risk of incident HF, progression, and death (33). Given the significant associations and biologic plausibility, these 2 biomarkers are candidates for further study.

In contrast to previous work, including our own when we had exclusively evaluated early increases in these markers (baseline to 3 months) (8), hs-cTnI and NT-proBNP did not show evidence of an association with cardiotoxicity and risk of first cardiotoxic event. In the results presented here, we cannot exclude the possibility that insufficient statistical power precluded us from detecting an effect, particularly since the hs-cTnI data were missing in a larger fraction of observations than the other biomarkers. Alternatively, for the cohort as a whole, hscTnI demonstrated a strikingly different pattern from the other biomarkers (Table 2, online Supplemental Fig. 1). hs-cTnI was substantially more increased at 3 months, and unlike other markers, this increase did not resolve by 15 months. We hypothesize that these increases in hscTnI might reflect processes that predispose patients to first cardiotoxicity, but that there is insufficient resolution of hs-cTnI increase in those who do not experience subsequent cardiotoxicity or who resolve cardiotoxicity to allow it to be an effective diagnostic biomarker at later time points. We also note that we used a hs-cTnI platform, in contrast to other studies (35). Clearly, our work is not definitive; there is a critical need for additional studies in larger cohorts and with extended follow-up time to further explore the role of hs-cTnI over the duration of chemotherapy.

There are a number of limitations. Our cohort was comprised of 78 patients, of whom 23 experienced ≥1 cardiotoxicity events. Overall, this number and particularly the number of individuals experiencing events are small. With alternative definitions of cardiotoxicity, we had only 11 events, further limiting power. The small number of outcomes limits our ability to differentiate between biomarker associations that are related to acute or delayed recovery, or lack thereof. Moreover, given the number of events and concerns of overfitting our models, we did not include potential confounders, such as medication use. The impact of confounding thus remains an important question for future studies. Last, our study was subject to incomplete data. We cannot rule out the possibility of bias due to these missing data, although our sensitivity analyses with the imputed data yielded results that were highly consistent with the analyses presented here. Although our work describes results for one of the largest cohorts in breast cancer with multiple and serial newer biomarker and echocardiographic measures, our findings require independent confirmation.

In conclusion, our results identify several biomarkers that may have clinical relevance to patients undergoing sequential therapy with doxorubicin and trastuzumab. In particular, assessment of MPO, perhaps in conjunction with PlGF and GDF-15, may improve on the identification of breast cancer patients who are at risk for cardiotoxicity with these agents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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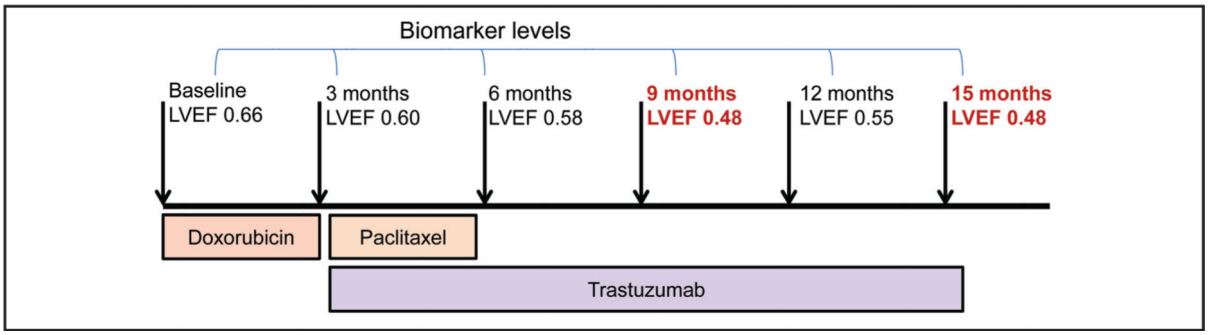

Fig. 1. Study protocol and analytic approach

Biomarkers, echocardiograms, and questionnaires were obtained at visits 1–6, at 0, 3, 6, 9, 12, and 15 months (vertical arrows). Biomarkers and cardiotoxicity status were defined at each visit; biomarkers were expressed as a ratio relative to baseline (tick marks). Example patient with cardiotoxicity at 9 and 15 months shown. Associations between biomarker ratios and cardiotoxicity were determined at each concurrent and subsequent visit.

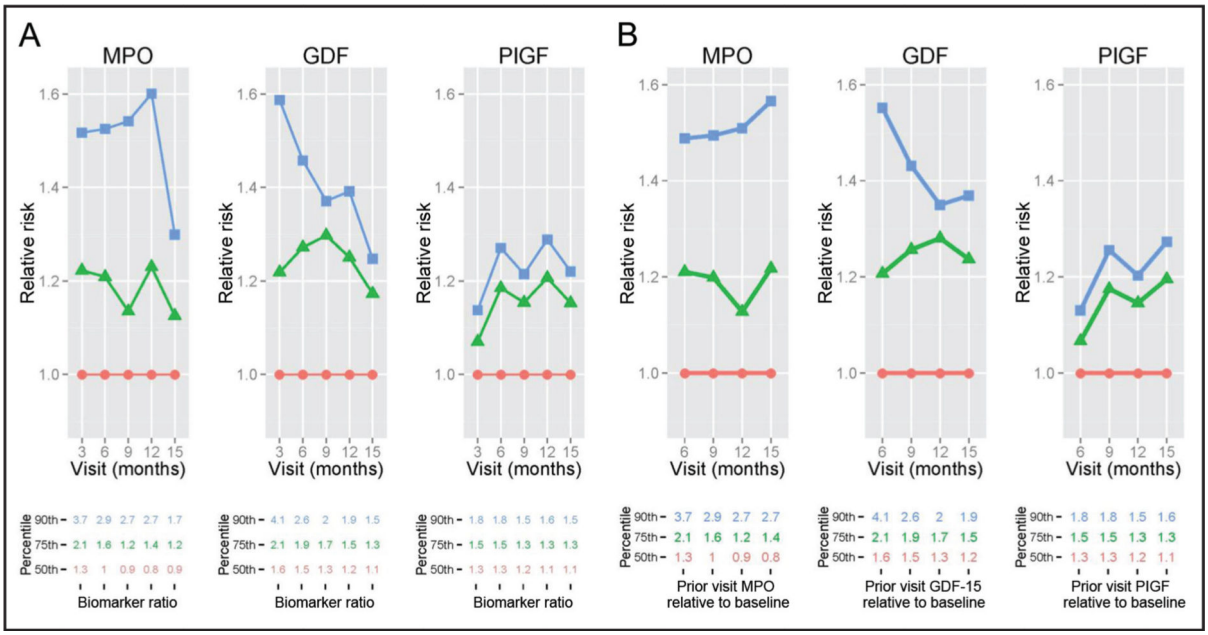

Fig. 2. Cardiotoxicity risk

Cardiotoxicity risk at each visit for the 75th (▲) and 90th (■) percentiles relative to the median (●) for biomarkers of primary interest as derived from multivariable models for the same visit (A) and subsequent visit (after 3 months) (B). The numbers below the graph indicate the biomarker ratio relative to baseline at the 50th, 75th, and 90th percentiles at the same visit (A) or the preceding visit (B).

Table 1

Baseline patient characteristics (n = 78).[a]

Characteristic	Value
Age, years	49 (10)
Body mass index, kg/m^2	25.5 (4.5)
Tobacco user	7 (9)
Breast cancer side	
Left	38 (49)
Bilateral	6 (8)
Hypertension	21 (27)
Diabetes	1 (1)
Hyperlipidemia	18 (23)
Radiotherapy	46 (60)
Angiotensin-converting enzyme inhibitor	13 (17)
Beta-blocker	10 (13)
LVEF, %	64 (5)

[a]Data are n (%) or mean (SD). Adapted with permission from Ky et al. (8).

Table 2

Biomarker levels at baseline and ratio of biomarkers relative to baseline at each visit.[a]

		Median biomarker level relative to baseline at indicated visit				
Biomarker	**Baseline (0 months)**	**3 months**	**6 months**	**9 months**	**12 months**	**15 months**
GDF-15, ng/L	500 (349-733)	**1.6**	**1.6**	**1.3**	**1.2**	**1.2**
MPO, pmol/L	107 (76.7-173)	**1.3**	1.0	0.9	0.8	0.9
PIGF, ng/L	19.6 (17.5-23.3)	**1.3**	**1.3**	**1.2**	**1.1**	**1.1**
hsCRP, mg/L	1.72 (0.633-3.92)	**1.6**	**1.4**	0.9	1.0	0.9
hsTnl, ng/L	1.30 (0.700-3.95)	**9.9**	**12.0**	**6.8**	**4.2**	**3.9**
Gal-3, μg/L	13.8 (10.8-18.2)	1.0	1.0	1.0	1.0	1.0
NT-proBNP, ng/L	71 (37.0-133)	0.9	0.7	1.0	1.1	1.2
sFlt-1, ng/L	236 (215-272)	**1.1**	**1.1**	1.0	1.0	1.0

[a]Data are median (interquartile range). Bold text indicates medians >1.0($P < 0.05$). In contrast to this table, Supplemental Figure 1 shows the log-transformed values as used in the models.

Table 3

Longitudinal associations between increases in biomarkers relative to baseline and cardiotoxicity.

	Risk of cardiotoxicity at the same visit				Risk ofcardiotoxicityat the subsequent visit			
	Univariable		Multivariable		Univariable		Multivariable	
Biomarker	**HR(95%CI)**	***P***	**HR(95%CI)**	***P***	**HR(95%CI)**	***P***	**HR(95%CI)**	***P***
GDF-15	1.80 (1.20-2.69)	0.007	2.16 (1.49-3.13)	<0.001	1.59 (1.06-2.40)	0.02	1.62 (1.07-2.44)	0.01
MPO	1.37 (1.11-1.69)	0.02	1.31 (1.08-1.60)	0.004	1.32 (1.11-1.58)	0.003	1.30 (1.06-1.58)	0.006
PIGF	3.77 (1.43-9.89)	0.04	3.09 (1.24-7.72)	0.008	2.61 (0.95-7.19)	0.08	3.27 (1.19-8.94)	0.01
hsCRP	1.18 (0.97-1.44)	0.07			1.12 (0.88-1.42)	0.19		
hsTnl	1.04 (0.91-1.20)	0.30			1.08 (0.96-1.20)	0.14		
GaI-3	1.31 (0.86-1.99)	0.14			1.60 (1.12-2.28)	0.04		
NT-proBNP	1.13 (0.86-1.49)	0.19			1.07 (0.83-1.38)	0.31		
sFIt-1	1.08 (0.54-2.16)	0.41			0.86 (0.49-1.50)	0.31		

Data are median (interquartile range) for a $\log_2$ ratio of the biomarker relative to baseline, i.e., for each doubling of the biomarker. All *P*-value shown are 1-sided.